# Simultaneous determination of multiclass emerging contaminants in aquatic plants by ultrasound-assisted matrix solid-phase dispersion and GC-MS

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**Abstract** A multiresidue method was developed for the simultaneous determination of 31 emerging contaminants (pharmaceutical compounds, hormones, personal care products, biocides, and flame retardants) in aquatic plants. Analytes were extracted by ultrasound-assisted matrix solid-phase dispersion (UA-MSPD) and determined by gas chromatography-mass spectrometry after sylilation, The method was validated for different aquatic plants (*Typha angustifolia*, *Arundo donax*, and *Lemna minor*) and a semi-aquatic cultivated plant (*Oryza sativa*) with good recoveries at concentrations of 100 and 25 ng $g^{-1}$ wet weight, ranging from 70 to 120 %, and low method detection limits (0.3 to 2.2 ng $g^{-1}$ wet weight). A significant difference of the chromatographic response was observed for some compounds in neat solvent versus matrix extracts, and therefore, quantification was carried out using matrix-matched standards in order to overcome this matrix effect. Aquatic plants taken from rivers located at three Spanish regions were analyzed, and the compounds detected were parabens, bisphenol A, benzophenone-3, cyfluthrin, and cypermethrin. The levels found ranged from 6 to 25 ng $g^{-1}$ wet weight except for cypermethrin that was detected at 235 ng $g^{-1}$ wet weight in *O. sativa* samples.

**Keywords** Aquatic plants · Ultrasound-assisted matrix solid-phase dispersion · GC-MS · Emerging contaminants · *Typha angustifolia* · *Oryza sativa* · Pharmaceuticals · Biocides

## Introduction

Emerging contaminants (ECs) have raised concern among the scientific community because of their ubiquitous presence in different environmental compartments (Aznar et al. 2014a; Wu et al. 2011; Bell et al. 2013; Wang et al. 2012) and the risk they may pose to the environment and human health, taking also into account their potential bioaccumulation in animals (Corcellas et al. 2015; Ortiz de Garcia et al. 2013; Wu et al. 2012) and uptake by plants (Tanoue et al. 2012), which are routes for entering the food chain (Calderon-Preciado et al. 2011; Rodil et al. 2012; Goldstein et al. 2014).

ECs (pharmaceuticals, personal care products, flame retardants, and biocides) are widely used and can reach the environment through effluent waters and the use of biosolids (Al Aukidy et al. 2012; Albero et al. 2012) or manure (Aznar et al. 2014a) applied to improve agricultural soils. Nevertheless, their continuous release through septic system effluents (Kinney et al. 2006) and discharge of wastewater treatment plants (WWTPs), after being inefficiently removed by conventional methods, are the main routes to reach different environmental compartments (Thomas and Foster 2005; Harada et al. 2008). Thus, ECs can be considered pseudo persistent, reaching the environment uninterruptedly and making them a priority to the scientific community.

Among biocide substances, synthetic pyrethroids (PYs), derived from chrysanthemic acid, are widely used indoors to control household insects, such as mosquitoes, termites, and other harmful insects, in place of more toxic organophosphorus and organochlorine insecticides. Indeed, they are much

more effective against a wide spectrum of pests than organochlorines and organophosphates and, consequently, they are used in many applications. However, several pyrethroids are known to affect the central nervous system of humans and to have endocrine-disrupting effects (Kohler and Triebskorn 2013). Moreover, PYs may have a negative impact on the environment, primarily on water bodies, due to their toxicity to arthropods (Wang et al. 2012; Weston et al. 2005; Song et al. 2015) and their bioaccumulation potential in fish (Corcellas et al. 2015). There are several processes involved in the contamination mitigation, such as hydrolysis, photolysis, adsorption, microbial degradation, and plant uptake. Several authors have addressed the ability of plants to uptake and translocate organic contaminants (Tanoue et al. 2012; Calderon-Preciado et al. 2011; Wu et al. 2013; Herklotz et al. 2010). Thus, aquatic plants, which are continuously exposed to organic contaminants transported by water, may be used as bioindicators of the occurrence of these contaminants in freshwater as well as for phytoremediation. Dordio et al. (2011a, b) showed that constructed wetland treatment of wastewaters showed removal efficiencies of 96, 97, and 75 % for ibuprofen, carbamazepine, and clofibric acid, respectively. Reinhold et al. (2010) reported on constructed treatment wetlands and demonstrated that they have the potential to reclaim wastewaters through the removal of trace concentrations of emerging organic pollutants, including personal care products, pharmaceuticals, and pesticides. These studies demonstrated that aquatic plants contributed both directly and indirectly to the aqueous depletion of emerging organic pollutants through both active and passive processes.

Multiresidue analyses of the organic pollutants in plants are limited by the complexity of the matrix and the properties of the analytes, requiring powerful and selective techniques such as gas chromatography (GC) coupled to mass spectrometry (MS) (Bisceglia et al. 2010; Niewiadowska et al. 2010) or LC-MS (Matamoros et al. 2012). GC-MS is a robust and less expensive technique generally available in most analytical laboratories, but the presence of polar functional groups with active hydrogens in some of the compounds studied requires the use of a derivatization procedure to reduce their polarity and enhance their volatility before GC analysis. Several authors have pointed out a gap of multiresidue methods for ECs in plants (Matamoros et al. 2012) and no multiresidue method has been found for the simultaneous analysis of multiclass ECs commonly found in freshwater, including pharmaceuticals, personal care products, flame retardants, and pyrethroids in aquatic plants.

Therefore, the aim of this study was to develop an analytical method, based on ultrasound-assisted matrix solid-phase dispersion (UA-MSPD) and gas chromatography-mass spectrometry (GC-MS), for the simultaneous determination of 31 organic pollutants (pharmaceuticals, personal care products, hormones, flame retardants, and biocides) in aquatic plants (*Typha angustifolia*, *Arundo donax*, *Oryza sativa*, and *Lemna minor*) and evaluate their presence in plants taken from three rivers in different Spanish regions.

## Material and methods

### Standards and reagents

Standards of bifenthrin, fenpropathrin, λ-cyhalothrin, permethrin, cyfluthrin, α-cypermethrin, τ-fluvalinate, esfenvalerate, deltamethrin, triclosan, and methyl triclosan (purity >99 %) were supplied by Riedel-de Haën (Seelze, Germany). Standards of estrone, hexestrol, diethylstilbestrol, ibuprofen, gemfibrozil, fenoprofen, naproxen, mefenamic acid, ketoprofen, carbamazepine, fenofibrate, nonylphenol, bisphenol A (BPA), benzophenone-3 (BP3), 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), 2,2′,4,4′,6-pentabromodiphenyl ether (BDE-100), methyl paraben and propyl paraben (purity >97 %) were purchased from Sigma-Aldrich (St Louis, MO, USA). Tris(2-carboxyethyl) phosphine (TCEP) and tris(2-chloroisopropyl) phosphate (TCPP) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). All target compounds are shown in Table 1.

Ethyl acetate (EtAc), acetonitrile (ACN), residue analysis grade, ammonium hydroxide ($NH_4OH$) ≥32 %, Silica Bondesil-C18, particle diameter of 40 μm, and primary secondary amine (PSA) (Bondesil-PSA, 40 μm) were purchased from Varian (Palo Alto, CA, USA). Enviro-Clean Bulk Chlorofiltr was acquired from Carlo Erba (Madrid, Spain). Graphitized carbon black (GCB) (Supelclean ENVI-Carb 120/400) was purchased from Supelco (Madrid, Spain). The derivatization agent *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA, purity ≥95 %) with 1 % *tert*-butyldimethylchlorosilane (TBDMCS) and formic acid were obtained from Sigma-Aldrich (St Louis, MO, USA). Florisil, 150–250 μm (60–100 mesh), was supplied by Aldrich (Steinheim, Germany). Magnesium sulfate anhydrous ($MgSO_4$) was purchased from Merck (Darmstadt, Germany).

Separate stock solutions of individual compounds were made up at 50 μg $mL^{-1}$ in ACN and stored at −18 °C. A mixed stock solution of 1 μg $mL^{-1}$ containing all analytes was prepared by dilution with ACN of the individual stock solutions. A working mixture solution at 500 ng $mL^{-1}$ was prepared weekly by dilution with ACN of the mixed stock solution. All solutions were stored in the darkness at 4 °C up to 8 weeks.

### Plant material

Samples of *T. angustifolia*, *A. donax*, and *L. minor* were provided by the School of Agricultural Engineering, Polytechnic University of Madrid, Spain. *O. sativa* was taken from the natural park of Albufera (Valencia, Spain) within an area

**Table 1** Physicochemical properties, retention times ($t_R$, min), and selected ions (m/z) of the compounds studied

| Name | Physicochemical properties[a] | | SIM parameters | | | |
|---|---|---|---|---|---|---|
| | Log $K_{ow}$ | pka | $t_R$ | *T* | *Q*1 | *Q*2 |
| Methyl paraben | 1.7 | 8.2 | 11.27 | 209 | 210 | 266 |
| TCEP | 1.6 | – | 11.38 | 249 | 250 | 63 |
| TCPP | 2.6 | – | 11.57 | 125 | 99 | 277 |
| Ibuprofen | 4 | 4.5 | 11.85 | 263 | 264 | 117 |
| Propyl paraben | 2.7 | 8.4 | 12.15 | 237 | 238 | 294 |
| Methyl triclosan | 5.2 | – | 13.38 | 302 | 304 | 252 |
| Nonylphenol | 3.8 | 10.7 | 13.38 | 334 | 277 | 278 |
| Gemfibrozil | 4.7 | 4.4 | 13.45 | 243 | 179 | 307 |
| Fenoprofen | 3.9 | 4.2 | 13.68 | 299 | 197 | 206 |
| BP3 | 3.7 | 7.6 | 14.11 | 285 | 242 | 286 |
| Naproxen | 3.2 | 4.8 | 14.13 | 287 | 185 | 288 |
| Triclosan | 4.8 | 8 | 14.34 | 347 | 345 | 200 |
| Mefenamic acid | 5.1 | 4.2 | 14.64 | 298 | 224 | 355 |
| Ketoprofen | 3.1 | 4.5 | 14.65 | 311 | 295 | 105 |
| Bifenthrin | 6 | – | 14.8 | 181 | 165 | 166 |
| Fenpropathrin | 6 | – | 14.87 | 125 | 181 | 265 |
| Carbamazepine | 2.5 | 13.9 | 14.95 | 193 | 194 | 293 |
| BDE-47 | 8.8 | – | 15.16 | 486 | 326 | 488 |
| Fenofibrate | 4.8 | −4.9 | 15.19 | 121 | 273 | 139 |
| λ-Cyhalothrin | 6.9 | – | 15.24 | 197 | 181 | 208 |
| Permethrin | 6.5 | – | 15.74 | 183 | 163 | 165 |
| BPA | 3.4 | 9 | 15.7 | 441 | 442 | 456 |
| BDE-100 | 8.9 | – | 15.92 | 404 | 406 | 566 |
| Cyfluthrin | 5.9 | – | 16.06 | 163 | 206 | 226 |
| Hexestrol | 4.8 | 9.9 | 16.35 | 249 | 250 | 337 |
| α-Cypermethrin | 6.6 | – | 16.41 | 163 | 165 | 181 |
| Diethylstilbestrol | 5.1 | – | 16.5 | 496 | 497 | 498 |
| τ-Fluvalinate | 4.3 | – | 17.09 | 250 | 252 | 181 |
| Estrone | 3.1 | 10.3 | 17.1 | 327 | 384 | 328 |
| Esfenvalerate | 4 | – | 17.2 | 125 | 167 | 181 |
| Deltamethrin | 6.1 | – | 17.74 | 181 | 253 | 251 |

*T* target ion, *Q1* and *Q2* qualifier ions

[a] Data compiled from previous studies: (Aznar et al. 2014b; Bhandari et al. 2009; Oros and Werner 2005)

irrigated with pure artesian water. Plants were maintained in darkness at 4 °C and transported to the laboratory where representative portions of the aerial part of the selected plants were ground using a food processor and kept at 20 °C until analyses. These samples, after preliminary screenings, did not show any of the target compounds included in this work and they were used as blanks. *T. angustifolia* was employed in the optimization of the method because it was the most complex matrix.

The validated method was applied to plants taken from rivers located at different Spanish regions (Valencia, Madrid, and Andalucía) during a sampling campaign carried out in summer 2015 to assess the presence of pollutants.

## Sample preparation

### *Ultrasound-assisted matrix solid-phase dispersion*

UA-MSPD was performed mixing 1 g of aquatic plant with 4 g of Florisil and 2 g of $MgSO_4$ in a glass mortar. Then the mixture was blended with a glass pestle for 5 min to yield a homogeneous material and placed in a 20-mL glass column (10 cm × 20 mm i.d., from Becton-Dickinson, Madrid, Spain) over two paper filters (Whatman No. 1 paper circles of 2-cm diameter, Maidstone, UK) at the end with 2 g of $MgSO_4$. EtAc with 3 % $NH_4OH$ (8 mL) was added to each column and 2 mL was used to wash the mortar and pestle. Columns were sonicated for 15 min in an ultrasonic water bath (Raypa, Barcelona, Spain) at room temperature. The water level in the bath was adjusted to equal the extraction solvent level inside the columns, which were supported upright in a tube rack and closed with one-way stopcocks. Then extracts were collected in tubes using a multiport vacuum manifold (Supelco, Visiprep, Madrid) and evaporated near dryness. The extraction was repeated twice with 5 mL ACN containing 4 % formic acid to ensure the complete extraction of the acidic target compounds. The extract was evaporated to 1 mL using a Genevac EZ-2 evaporator (purchased from NET Interlab, Spain) before the cleanup step.

### *Cleanup*

Aquatic plant extracts (1 mL) were cleaned through a 5-mL glass column (Normax, Lisbon, Portugal) with two paper filters (Whatman No. 1, Maidstone, UK) containing 1 g of $MgSO_4$ and 1 g of C18. Analytes were eluted with 5 mL of ACN and extracts were collected in tubes using a multiport vacuum manifold, evaporated to dryness, and reconstituted to 0.5 mL with ACN before their derivatization.

### *Derivatization*

Prior to the GC-MS determination, some of the studied analytes need to be derivatized to increase their volatility. The derivation agent MTBSTFA:TBDMCS (99:1, *v*/*v*) was selected, as it presents the best performance for pharmaceutical and personal care products in comparison with other derivatization agents (Schummer et al. 2009). Thus, the *t*-butyldimethylsilyl derivatives were prepared by the addition of 50 μL of MTBSTFA:TBDMCS (99:1, *v*/*v*) to an aliquot (100 μL) of the plant extract and transferred into a 2-mL reaction vial with a micro insert. Vials were closed and the mixture was left to react for 1 h at 70 °C before the GC-MS analysis.

### Detection equipment

Gas chromatography-mass spectrometry (GC-MS) analysis was performed with an Agilent 6890 (Waldbronn, Germany) gas chromatograph equipped with an automatic injector and a mass spectrometric detector, model HP 5977A. A fused silica capillary column ZB-5MS, 5 % phenyl polysiloxane as nonpolar stationary phase (30 m × 0.25 mm i.d. and 0.25-μm film thickness), from Phenomenex (Torrance, CA), was used for the analysis.

Operating conditions were in solvent-vent mode as follows: 2 μL of plant extracts were injected in a simple-taper glass liner with a nominal volume of 800 μL with glass wool. The split vent was open for 0.1 min with an inlet pressure of 5 psi and a flow rate of 100 mL $min^{-1}$. Once the sample introduction was completed, the inlet was switched to splitless mode for analyte transfer. After 2.6 min, the purge value was activated at a 60 mL $min^{-1}$ flow rate. Helium (purity 99.995 %) was used as carrier gas at a constant flow rate of 1.2 mL $min^{-1}$. The temperature program of the oven started at 50 °C, kept for 0.1 min, then ramped to 300 °C at 600 °C $min^{-1}$, held 5 min, and finally decreased to the initial temperature cooling with compressed air. The column temperature was maintained at 50 °C for 2.6 min, then programmed at 20 °C $min^{-1}$ to 300 °C and held for 5 min. The total analysis time was 20.1 min and the equilibration time was 4 min.

The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 eV, an ion source temperature of 230 °C, and a quadrupole temperature of 150 °C. The electron multiplier voltage was set with a gain factor of 4 and a solvent delay of 10.5 min was used. Table 1 lists the compounds with their retention times and selected ions to be used in SIM mode. The target and qualifier abundances were determined by injection of standards under the same chromatographic conditions using full scan with the mass/charge ratio ranging from 50 to 550 m/z. The compounds were confirmed by their retention times, the identification of target and qualifier ions, and the determination of qualifier to target ratios. Retention times must be within ±0.1 min of the expected time and qualifier-to-target ratios within a 20 % range for positive confirmation. The quantification was accomplished by matrix-matched calibration.

### Method validation

In order to evaluate the method developed for the detection of ECs in aquatic plants, different quality parameters were studied: recoveries, precision, linearity, and sensitivity.

Plants were spiked with the analytes at two levels (25 and 100 ng $g^{-1}$) with four sample replicates, to study the recoveries and the accuracy of the method. The precision of the method was evaluated in terms of repeatability (intra-day precision) and reproducibility (inter-day precision) at 100 and 25 ng $g^{-1}$. The repeatability was assessed by the application of the whole procedure on the same day, and the reproducibility was evaluated performing the complete procedure in different days. The results were expressed as %RSD (six replicates).

To evaluate the sensitivity, method detection limit (MDL) and limit of quantification (LOQ) of the developed method were determined using ten replicates of plant extracts, spiked at 2.5 ng $g^{-1}$. The equation to calculate the MDL was the following: $MDL = t_{99} \times S$, where $t_{99}$ is the Student value for a 99 % confidence level and $n$-1 degrees of freedom and $S$ is the standard deviation of the replicate analyses. The LOQ was calculated as 10 times the standard deviation of the results of the replicate analysis used to determine MDL.

Finally, to evaluate linearity and matrix effect, two sets of calibration solutions were prepared in the range from 1 to 400 ng $g^{-1}$; one set was solvent-based and the other was prepared spiking blank plant extracts at the same concentrations. There are several approaches to counteract matrix-induced effects, but due to the high price and the nonexistence of internal standards for some of the 31 organic pollutants studied, matrix-matched calibration was selected (Aznar et al. 2014b).

In order to reduce possible memory effects of the column, prior to the analysis of samples, the inlet was flushed by heating at 300 °C for 30 min and one laboratory blank was run with each set of samples to check for memory effects and demonstrate laboratory background levels.

### Statistical analysis

Standard statistical analyses were carried out to study the significant differences of the method using STATGRAPHIC CENTURION. One-way ANOVA was applied to determine significant differences at a $p \leq 0.01$ level.

## Results and discussion

### Solvent selection

In the first assay, 1 g of *T. angustifolia* spiked at 100 ng $g^{-1}$ was placed in a mortar and blended with 4 g of Florisil and 2 g of $MgSO_4$. Different organic solvents and combination of them changing the pH were tested to evaluate extraction yields. Figure 1 shows the recoveries obtained with different extraction solvents for a representative selection of the studied compounds. Firstly, two extractions with ACN did not present good recoveries, particularly for the acidic compounds (i.e., mefenamic acid, data not shown). Thus, the pH of the second extraction was changed (adding 3 % of formic acid), and the recoveries of those compounds clearly improved. In order to improve the extraction yields of the target analytes, particularly the more lipophilic compounds, such as pyrethroids and the brominated flame retardants (BDE-47 and BDE-100), a less polar solvent was selected to perform the first step of the

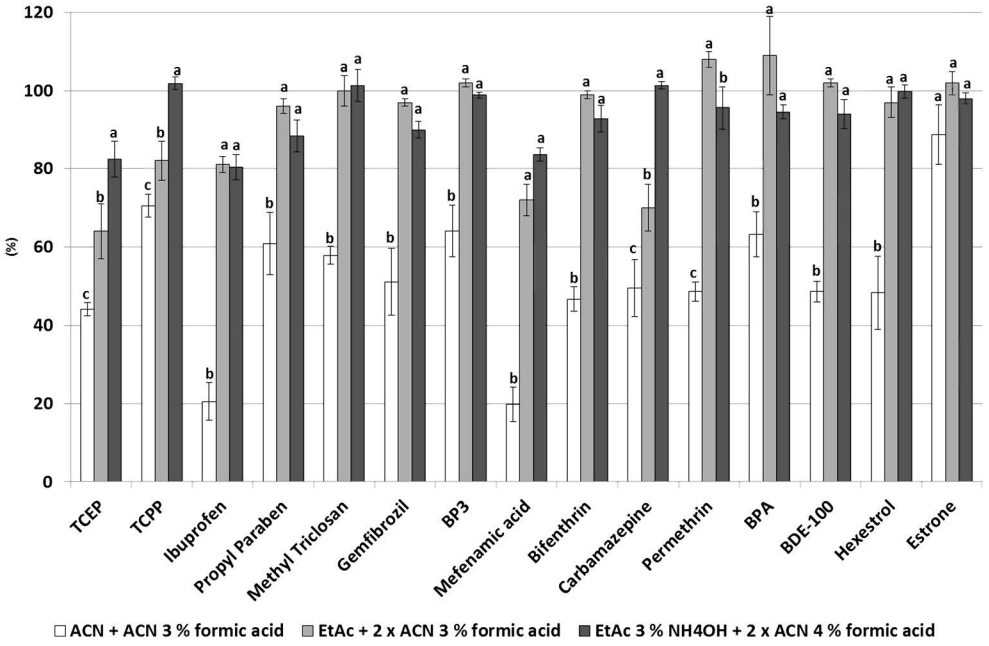


**Fig. 1** Solvent selection using *Typha angustifolia* spiked at 100 ng $g^{-1}$. Different *letters* indicate significant differences ($p \leq 0.01$)

procedure; therefore, the extraction with EtAc and ACN containing 3 % formic acid was carried out, but yields of basic compounds (i.e., carbamazepine) (Table 1) were low and an extraction with basic solvent was required. The best performance was reached using EtAc with 3 % $NH_4OH$ and two extractions with ACN containing 4 % formic acid as can be seen in Fig. 1. The addition of formic acid resulted in recoveries around 100 % for the acid compounds, and the use of $NH_4OH$ during the first extraction improved the recovery of the basic compounds, particularly for carbamazepine. Moreover, significant differences ($p \leq 0.01$) were found in the statistical analysis of the selection of extraction solvent and the best recoveries were obtained with EtAc 3 % $NH_4OH + 2 \times$ ACN 4 % formic acid, as shown in Fig. 1.

## Cleanup

Although target analytes can be determined in the conditions indicated above, plant extracts showed high content of chlorophyll and some interference appeared during MS quantification; for these reasons a cleanup step was necessary. Dispersive solid-phase extraction (dSPE) was carried out with 1 mL blank extracts spiked at 100 ng $g^{-1}$ and 0.1 g of sorbent. Four sorbents were tested: GCB, PSA, Chlorofiltr, and C18.

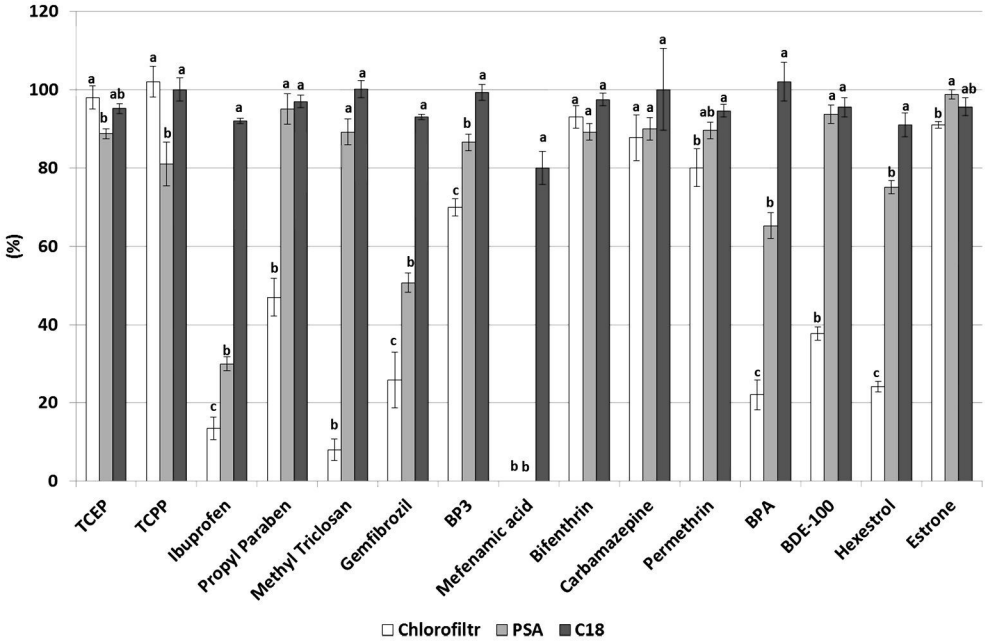


**Fig. 2** Cleanup sorbent selection using *Typha angustifolia* spiked at 100 ng $g^{-1}$. Different *letters* indicate significant differences ($p \leq 0.01$)

GCB showed the best performance removing chlorophylls completely, but it showed the lowest recoveries because planar compounds are retained in its surface (data not shown). PSA removes pigments and sugars, but showed the poorest removal of green pigments, and all the acidic compounds studied were not recovered at all because of the amine group of the PSA, as described by other authors (Paya et al. 2007). Recoveries using Chlorofiltr were much higher, but the extracts remained slightly green, although its main purpose is to eliminate chlorophyll. C18 showed the best performance eliminating most of the chlorophyll and other interferences with the best recoveries as shown in Fig. 2. Thus, C18 was selected as sorbent for the purification of the extracts using 5-mL glass columns. The influence of the amount of C18 (0.5, 1, or 2 g) was also studied and 0.5 g were not enough to remove interferences but with 1 and 2 g, similar recoveries and removal of interferences were obtained. Therefore, the cleanup was performed with 1 g of C18.

## Method validation

In order to determine the accuracy of the method, recoveries were carried out spiking four different aquatic plants at two levels, 100 and 25 ng $g^{-1}$ (Table 2). Satisfactory recoveries were obtained with the four plants for most of the compounds. In comparison to other studies in plants (Wu et al. 2012; Winker et al. 2010), where less compounds were studied, our work showed in general better recoveries. Repeatability was evaluated by analyzing six replicates within a given day and reproducibility by determining the recoveries of six

**Table 2** Recoveries (%) and relative standard deviations (RSD ($n = 4$), % in parenthesis) obtained for target compounds in different aquatic plants

| | *Typha angustifolia* | | *Arundo donax* | | *Lemna minor* | | *Oryza sativa* | |
|---|---|---|---|---|---|---|---|---|
| | 100 ng $g^{-1}$ | 25 ng $g^{-1}$ | 100 ng $g^{-1}$ | 25 ng $g^{-1}$ | 100 ng $g^{-1}$ | 25 ng $g^{-1}$ | 100 ng $g^{-1}$ | 25 ng $g^{-1}$ |
| Methyl paraben | 70 (8) | 82 (8) | 76 (5) | 72 (9) | 83 (5) | 88 (8) | 75 (5) | 81 (6) |
| TCEP | 84 (4) | 80 (9) | 74 (3) | 94 (6) | 94 (7) | 85 (6) | 90 (3) | 73 (3) |
| TCPP | 94 (9) | 81 (8) | 100 (3) | 96 (9) | 83 (8) | 91 (5) | 99 (3) | 74 (6) |
| Ibuprofen | 100 (3) | 71 (3) | 70 (6) | 72 (5) | 75 (2) | 73 (3) | 120 (7) | 112 (9) |
| Propyl paraben | 89 (3) | 80 (6) | 86 (2) | 80 (5) | 88 (2) | 90 (4) | 78 (6) | 74 (10) |
| Methyl triclosan | 88 (3) | 74 (7) | 87 (4) | 83 (8) | 111 (3) | 79 (4) | 86 (4) | 73 (5) |
| Gemfibrozil | 86 (6) | 81 (5) | 77 (7) | 79 (10) | 77 (3) | 77 (8) | 81 (9) | 71 (4) |
| Nonylphenol | 87 (2) | 83 (8) | 70 (2) | 77 (5) | 88 (3) | 79 (4) | 98 (2) | 80 (3) |
| Fenoprofen | 78 (9) | 71 (3) | 72 (8) | 78 (2) | 78 (3) | 81 (3) | 87 (5) | 74 (6) |
| BP3 | 97 (4) | 98 (6) | 78 (7) | 80 (4) | 79 (3) | 76 (4) | 120 (5) | 71 (6) |
| Naproxen | 82 (8) | 98 (2) | 86 (3) | 79 (5) | 81 (4) | 82 (9) | 99 (3) | 75 (2) |
| Triclosan | 89 (4) | 84 (2) | 94 (11) | 80 (7) | 84 (2) | 85 (5) | 99 (2) | 78 (3) |
| Mefenamic acid | 91 (8) | 94 (8) | 70 (9) | 107 (3) | 74 (8) | 87 (4) | 80 (8) | 79 (3) |
| Ketoprofen | 73 (2) | 120 (2) | 75 (10) | 98 (8) | 72 (4) | 100 (3) | 98 (9) | 73 (2) |
| Bifenthrin | 86 (3) | 89 (7) | 94 (5) | 82 (5) | 91 (4) | 93 (3) | 88 (6) | 75 (4) |
| Fenpropathrin | 84 (3) | 89 (7) | 90 (6) | 73 (5) | 97 (9) | 95 (9) | 95 (4) | 71 (2) |
| Carbamazepine | 90 (2) | 83 (2) | 88 (3) | 75 (9) | 98 (2) | 96 (4) | 85 (5) | 79 (3) |
| BDE-47 | 76 (8) | 84 (9) | 89 (3) | 82 (6) | 86 (5) | 88 (6) | 90 (4) | 74 (3) |
| Fenofibrate | 83 (3) | 82 (8) | 89 (5) | 83 (5) | 87 (5) | 91 (6) | 94 (3) | 80 (4) |
| λ-Cyhalothrin | 83 (11) | 80 (5) | 85 (6) | 90 (11) | 93 (6) | 82 (7) | 91 (3) | 101 (8) |
| Permethrin | 83 (2) | 83 (8) | 91 (5) | 79 (6) | 89 (6) | 83 (3) | 92 (5) | 86 (5) |
| BPA | 77 (7) | 77 (4) | 107 (10) | 105 (3) | 80 (10) | 80 (4) | 97 (5) | 70 (5) |
| BDE-100 | 86 (2) | 80 (9) | 88 (4) | 79 (9) | 86 (8) | 87 (4) | 91 (4) | 86 (4) |
| Cyfluthrin | 80 (4) | 84 (7) | 87 (5) | 82 (8) | 82 (9) | 88 (7) | 90 (7) | 96 (6) |
| Hexestrol | 90 (0) | 80 (7) | 85 (3) | 78 (6) | 92 (2) | 100 (2) | 99 (2) | 77 (4) |
| α-Cypermethrin | 81 (3) | 79 (8) | 83 (4) | 87 (10) | 87 (8) | 89 (7) | 91 (3) | 95 (8) |
| Diethylstilbestrol | 97 (6) | 101 (2) | 111 (4) | 86 (6) | 84 (3) | 72 (3) | 77 (2) | 72 (3) |
| τ-Fluvalinate | 74 (3) | 85 (8) | 82 (6) | 86 (6) | 76 (7) | 87 (8) | 86 (5) | 92 (9) |
| Estrone | 103 (11) | 97 (9) | 95 (5) | 74 (10) | 98 (3) | 81 (3) | 107 (9) | 89 (7) |
| Esfenvalerate | 82 (11) | 74 (6) | 95 (6) | 89 (2) | 80 (5) | 83 (6) | 95 (7) | 92 (8) |
| Deltamethrin | 80 (5) | 73 (11) | 83 (7) | 95 (7) | 83 (6) | 90 (7) | 85 (8) | 89 (5) |

replicates within different days, and RSD lower than 6 and 10 %, respectively, was obtained.

MDLs and LOQs obtained for the different aquatic plants are shown in Table 3. MDLs ranged from 0.3 to 2.2 ng $g^{-1}$. The differences reported were related to the background noise and the recoveries obtained in the four matrices. Low limits were achieved for all the aquatic plants, being similar to those reported by Wu et al. (2012) in vegetables and better than the ones published by other authors (Winker et al. 2010; Calderon-Preciado et al. 2009).

The chromatographic response of analytes may be affected by the presence of matrix components. Therefore, matrix effects were evaluated preparing two multipoint calibration curves using a set prepared with solvent-based standards and the other with matrix-matched standards. A good linearity was obtained in the range of 1 to 400 ng $g^{-1}$, with correlation coefficients ≥0.994 for all the compounds studied. The slopes obtained by plotting the seven concentration levels against response, following linear regression analysis, were compared (Fig. 3). A significant difference of the chromatographic response was observed for bifenthrin (response increase) and for carbamazepine (response suppression). On the other hand, compounds such as diethylstilbestrol were not affected by matrix components. The analysis of pharmaceuticals in neat solvent produced, in general, calibration curves with higher slopes when compared to matrix-matched standards. Hormones did not show differences and the analysis of pyrethroids in neat solvent produced calibration curves with lower slopes when compared to matrix-matched standards for most compounds. In general, an enhancement of analyte response is

**Table 3** Method detection limits (MDL, ng $g^{-1}$) and limits of quantification (LOQ, ng $g^{-1}$) obtained for the analytes in the different aquatic plants studied

| | *Typha angustifolia* | | *Arundo donax* | | *Lemna minor* | | *Oryza sativa* | |
|---|---|---|---|---|---|---|---|---|
| | MDL | LOQ | MDL | LOQ | MDL | LOQ | MDL | LOQ |
| Methyl paraben | 0.4 | 1.2 | 0.5 | 1.7 | 0.4 | 1.5 | 0.8 | 3.0 |
| TCEP | 1.5 | 3.2 | 0.5 | 1.9 | 0.6 | 2.1 | 1.2 | 4.2 |
| TCPP | 0.8 | 2.4 | 0.3 | 1.0 | 0.9 | 2.9 | 0.8 | 2.8 |
| Ibuprofen | 0.8 | 2.7 | 0.7 | 2.6 | 0.8 | 2.7 | 0.8 | 2.8 |
| Propyl paraben | 0.3 | 1.0 | 0.3 | 1.0 | 0.3 | 1.2 | 1.1 | 3.8 |
| Methyl triclosan | 1.3 | 4.0 | 0.3 | 1.0 | 0.5 | 1.6 | 1.5 | 5.3 |
| Gemfibrozil | 0.5 | 1.5 | 1.1 | 3.2 | 0.3 | 1.1 | 0.8 | 2.7 |
| Nonylphenol | 0.4 | 1.5 | 1.2 | 3.8 | 1.4 | 3.8 | 0.7 | 2.5 |
| Fenoprofen | 0.9 | 2.6 | 1.3 | 3.7 | 0.6 | 2.0 | 1.0 | 2.9 |
| BP3 | 0.8 | 2.5 | 1.0 | 3.2 | 0.8 | 2.7 | 0.4 | 1.4 |
| Naproxen | 1.0 | 3.1 | 1.0 | 3.1 | 0.7 | 2.4 | 1.0 | 3.3 |
| Triclosan | 0.3 | 1.0 | 1.0 | 3.5 | 0.6 | 1.9 | 0.6 | 1.9 |
| Mefenamic acid | 0.4 | 1.4 | 0.9 | 2.7 | 0.8 | 2.5 | 0.9 | 3.1 |
| Ketoprofen | 0.3 | 1.0 | 1.5 | 4.8 | 1.1 | 3.2 | 2.1 | 6.3 |
| Bifenthrin | 0.4 | 1.3 | 0.3 | 1.0 | 0.5 | 1.9 | 0.7 | 2.5 |
| Fenpropathrin | 1.2 | 3.6 | 0.3 | 1.0 | 0.5 | 1.6 | 0.6 | 2.1 |
| Carbamazepine | 0.5 | 1.5 | 0.7 | 2.4 | 0.3 | 1.1 | 0.3 | 1.0 |
| BDE-47 | 0.7 | 2.5 | 1.1 | 3.8 | 0.8 | 2.6 | 0.3 | 1.0 |
| Fenofibrate | 1.0 | 2.7 | 0.7 | 2.3 | 0.7 | 2.0 | 0.5 | 1.6 |
| λ-Cyhalothrin | 0.9 | 3.0 | 0.8 | 2.8 | 0.9 | 3.1 | 0.8 | 2.7 |
| Permethrin | 1.1 | 3.6 | 0.3 | 1.0 | 0.5 | 1.8 | 0.7 | 2.3 |
| BPA | 1.5 | 3.2 | 1.1 | 3.4 | 0.6 | 1.9 | 0.9 | 3.0 |
| BDE-100 | 1.1 | 3.6 | 0.4 | 1.3 | 1.0 | 3.5 | 0.3 | 1.0 |
| Cyfluthrin | 1.0 | 3.1 | 0.6 | 2.1 | 0.8 | 2.6 | 0.3 | 1.0 |
| Hexestrol | 0.5 | 1.8 | 0.4 | 1.3 | 0.4 | 1.3 | 0.9 | 3.1 |
| α-Cypermethrin | 1.1 | 3.5 | 0.3 | 1.0 | 0.8 | 2.7 | 0.9 | 3.1 |
| Diethylstilbestrol | 0.3 | 1.0 | 0.9 | 3.0 | 0.7 | 2.4 | 2.2 | 6.7 |
| τ-Fluvalinate | 0.9 | 2.8 | 0.6 | 2.0 | 0.6 | 2.0 | 0.4 | 1.2 |
| Estrone | 0.5 | 1.8 | 0.9 | 3.1 | 0.7 | 2.5 | 0.4 | 1.2 |
| Esfenvalerate | 0.6 | 2.0 | 0.8 | 2.8 | 1.1 | 3.7 | 1.4 | 4.2 |
| Deltamethrin | 1.3 | 3.3 | 0.6 | 2.2 | 2.2 | 4.8 | 1.2 | 4.0 |

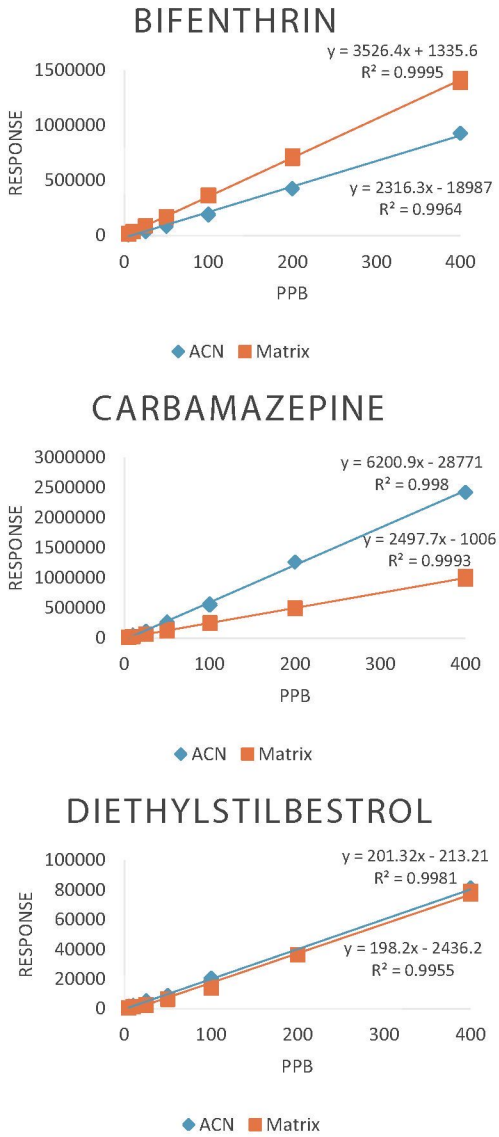


**Fig. 3** Comparison of calibration curves of bifenthrin, carbamazepine, and diethylstilbestrol, obtained by injection of standards in neat solvent (*diamond*) and spiked plant extracts (*square*)

observed in gas chromatography due to the presence of co-extractives that may improve the transfer of some compounds, i.e., pyrethroids, by blocking active sites in the chromatographic system. On the other hand, although the derivatization step is carried out with an excess of reagent, matrix components may compete with target analytes, and thus, a lower chromatographic response is observed, as it occurs in general with pharmaceuticals. Thus, quantification was carried out using matrix-matched standards in order to overcome the matrix effects observed and have more accuracy in the quantification of samples.

## Analysis of real samples

The developed analytical method was applied to different aquatic plants collected from rivers located in three Spanish regions (Valencia, Madrid, and Andalucía) in order to show the feasibility of the analytical technique to detect environmental levels of the selected contaminants in common aquatic plants of the studied area.

Table 4 shows the compounds found in the different aquatic plants analyzed. Methyl and propyl paraben, BPA, BP3, cyfluthrin, and cypermethrin were found at levels ranging from 6 to 25 ng $g^{-1}$ wet weight, except for cypermethrin that was detected at 235 ng $g^{-1}$ wet weight in *O. sativa* samples.

The concentration and fate of emerging contaminants in water, where several processes like adsorption, transport, and degradation are involved, affect their bioavailability to plants. In the studied area of Turia and Manzanares rivers (Valencia and Madrid, respectively), methyl and propyl paraben, BPA, triclosan, and nonylphenol were often detected in water (Carmona et al. 2014; Esteban et al. 2014). On the contrary, no residues were reported in the area of Guadalfeo river (Andalucia), which is located in a sparsely populated area with scarce industrial activity and, consequently, no residues were found in the aquatic plants from this river. Among

**Table 4** Analytes detected in aquatic plants

| | Turia River (Valencia) | | | Albufera Lake (Valencia) | Manzanares River (Madrid) | | Guadalfeo River (Andalucía) |
|---|---|---|---|---|---|---|---|
| | *Typha angustifolia* | *Arundo donax* | *Lemna minor* | *Oryza sativa* | *Typha angustifolia* | *Arundo donax* | *Arundo donax* |
| Methyl paraben | n.d. | n.d. | n.d. | n.d. | 12 ± 1 | n.d. | n.d. |
| Propyl paraben | 7 ± 1 | n.d. | n.d. | 14 ± 1 | n.d. | n.d. | n.d. |
| BP3 | 8 ± 1 | n.d. | n.d. | n.d. | 17 ± 6 | 20 ± 2 | n.d. |
| BPA | 15 ± 1 | 18 ± 3 | 18 ± 2 | 25 ± 6 | n.d. | n.d. | n.d. |
| Cyfluthrin | 6 ± 1 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Cypermethrin | n.d. | n.d. | n.d. | 235 ± 19 | n.d. | n.d. | n.d. |

Data are expressed as nanogram per gram wet weight, mean ± SD for $n = 3$

*n.d* not detected

the compounds most frequently detected in water, BPA and methyl and propyl paraben, were also detected in aquatic plants, whereas triclosan and nonylphenol were not found, although they have been reported at higher levels in water from the studied area (Carmona et al. 2014; Esteban et al. 2014). A greater translocation has been reported for compounds with log $K_{ow}$ between 1 and 3.5, whereas compounds with higher log $K_{ow}$ tend to be not taken up (Eggen et al. 2013; García-Valcárcel et al. 2016). This may explain why triclosan and nonylphenol were not detected in the aquatic plants studied (see Table 1).

Cypermethrin was detected in *O. sativa* collected from Albufera (Valencia) that is fed by the Turia River. In this river, cypermethrin was found in water and fish due to the intense agricultural use of the surroundings (Ccanccapa et al. 2016; Corcellas et al. 2015).

## Conclusions

A multiresidue method was developed and validated for the determination of 31 organic pollutants in aquatic plants. UA-MSPD was applied to extract target compounds from *T. angustifolia*, *A. donax*, *O. sativa*, and *L. minor* at two levels (100 and 25 ng $g^{-1}$ wet weight). These contaminants were determined by gas chromatography-mass spectrometry after reaction with MTBSTFA:TBDMCS to derivatize amine and hydroxyl polar groups of the target analytes. Good recoveries were obtained for most of the compounds in the four aquatic plants studied, with low limits of detection and quantification. The developed method was applied to aquatic plants collected from three rivers located in different Spanish regions to demonstrate the feasibility of the analytical technique to detect the selected contaminants in common aquatic plants at environmental levels. Six of the compounds studied were detected at concentrations ranging from 6 to 25 ng $g^{-1}$ wet weight, except cypermethrin that was detected at 235 ng $g^{-1}$ wet weight in *O. sativa* samples. Given the diverse physical-chemical properties of the compounds considered in the present study, this method can be used for monitoring other organic contaminants in aquatic plants. Nevertheless, further research needs to be done on the use of the developed analytical technique to assess the contamination of rivers and to better understand the role that aquatic plants may play regarding pollution mitigation in aquatic environments such as wetlands and rivers.

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